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The binary complex of pig plasma gelsolin with Mg^{2+}-G-actin in ATP and ADP

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Pig plasma gelsolin combined with Mg-G-actin at $<10^{-8}$ M Ca^{2+} to yield a binary complex. Complexes formed from G-actin with bound ATP or ADP. They contained approx. 1 mol of non-exchangeable nucleotide per mol of actin. ATP hydrolysis was not coupled to binary complex formation, but ATP in the complex hydrolysed very slowly. The nucleotide in the binary complex behaved like one of the two nucleotide molecules in the ternary complex (two actin monomers to one gelsolin), but the actin-gelsolin interaction was weaker in the binary complex.

Actin; Gelsolin; ATP; Ca^{2+}-sensitive protein; Actin-binding protein

1. INTRODUCTION

Gelsolins form complexes with actin monomers. A ternary complex of one plasma gelsolin molecule with two actins (GA_2) forms at greater than micromolar Ca^{2+} [1–6]. A binary complex (GA′) may be prepared by treatment of GA_2 with EGTA [4,7,8], which releases one actin monomer. This binary complex contains trapped calcium ions. Pig plasma gelsolin interacts directly with actin at low calcium ion concentrations to form a binary complex [5] which is Ca^{2+}-free (GA*) (Weeds, A., personal communication). The actin in this complex is stabilized by Mg^{2+}, rather than Ca^{2+}, at the high-affinity cation site [9–11].

The behaviour of actin-bound nucleotide in the Ca^{2+}-free GA* is described here and compared with that of nucleotide in GA_2, also prepared from Mg^{2+}-actin.

2. MATERIALS AND METHODS

2.1. *Materials*

[2,8-^{3}H]ADP and [2,8-^{3}H]ATP (ammonium salts) from Amersham International were stored at −20°C. Both were over 90% pure. Creatine phosphate, creatine phosphokinase and hexokinase (Type F300) were obtained from Sigma. Free Ca^{2+} concentrations in Ca^{2+}-EGTA buffers were calculated according to the dissociation constants given in [12].

Pig plasma gelsolin was purified as [13]. Rabbit skeletal muscle actin was prepared according to [14]. Mg-G-actin was prepared by exhaustive dialysis of F-actin at <5 mg·ml^{-1} against 2 mM Tris-Cl, pH 8.0, 0.2 mM ATP, 0.2 mM dithiothreitol, 50 μM $MgCl_2$, 0.1 mM EGTA, 1 mM sodium azide, followed by 30 min centrifugation in a Beckman airfuge. Equilibration with [^{3}H]ATP was complete in <5 min (measured as in [15]) giving 1.1 mol nucleotide per mol of actin. Mg-G-actin was routinely incubated with [^{3}H]ATP for 30–60 min before experiments with creatine phosphate (2 mM) and creatine phosphokinase (0.1 mg·ml^{-1}) present as an ATP-regenerating system. G-actin-ADP was prepared using hexokinase and glucose to deplete ATP [16–18]; >94% of the nucleotide was converted to ADP by this treatment. Exchange of [^{3}H]ADP onto G-actin in 2 mM $MgCl_2$, 100 mM KCl, pH 7 [15], was complete in <30 min giving 1.1 ± 0.2 SD (n = 3) mol ADP per mol actin. For the binary complex (GA*), gelsolin and G-actin were incubated at between 1 and 7 μM of each component in 5 mM imidazole-Cl, pH 7.0, 150 mM NaCl, 2 mM $MgCl_2$, 0.1 mM EGTA, 1 mM sodium azide, for 16–24 h.

2.2. *High-performance liquid chromatography*

HPLC was carried out on a TSK G4000SW column (Chrompak) with a flow rate of 0.5 ml·min^{-1}. Sample size was 50–500 μl. 0.5 ml fractions were collected and appropriate aliquots counted in a Packard Tricarb liquid scintillation spectrometer.

2.3. *Nucleotide binding and nucleotide analysis*

Nucleotide binding to proteins was determined after removal

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of free nucleotide by Dowex-1-8XCl [19]. The ATP and ADP contents of samples were analysed as previously [20,21].

2.4. *Protein quantitation*

SDS-PAGE was performed on 10% 10 × 10 cm slab gels [22] using a discontinuous buffer system [23]. Protein composition of complexes was determined by densitometry of Coomassie blue-stained gels. Equal staining of actin and gelsolin was assumed [3]. Protein concentrations were determined spectrophotometrically using an A_{290} of 0.63 for 1 mg·ml^{-1} of G-actin [24] and A_{280} of 1.24 for 1 mg·ml^{-1} of plasma gelsolin [13]. Absorbances of GA and GA_2 were calculated using A_{280} for G-actin 1.1 (1 mg·ml^{-1}) [25]; complex absorbance was assumed to equal the sum of the absorbances of the component proteins. The M_r of G-actin was taken as 42000 [26] and that of plasma gelsolin as 83000 [27].

3. RESULTS

3.1. *GA* formation*

GA* was isolated by HPLC. In excess EGTA (free $Ca^{2+} < 10^{-8}$ M) a single complex peak was formed, identified as GA* by SDS-PAGE (actin:gelsolin 1.1 ± 0.2 SD, $n = 4$) (fig.1A). There was no formation of larger complexes, even in up to four-fold actin excess. GA* was routinely prepared from close to equimolar mixtures of gelsolin and actin. Complex formation required approximately physiological ionic strength. At lower ionic strength (e.g. actin depolymerizing conditions) only about half as much GA* formed in 24 h.

GA* formed from either Mg-G-actin-ATP or Mg-G-actin-ADP. Formation was tested below the critical concentration for Mg-G-actin-ADP [18] and occurred approximately as efficiently at 1.1 μM actin as at 5 μM actin.

3.2. *Nucleotide binding, hydrolysis and exchange by GA**

GA* prepared at 6 μM contained 1.04 ± 0.07 ($n = 3$) mol nucleotide per mol of complex. Nucleotide cleavage was tested after mixing gelsolin and actin 1:1. After 10 min, when >50% formation of GA* is expected (Harris, H., unpublished), 86% of the protein-bound nucleotide was ATP, equivalent to that in the actin control. Thus there was no cleavage of nucleotide as GA* formed.

GA* complex containing 73% of its bound nucleotide as ATP was incubated overnight in the presence of free nucleotide and an ATP-regenerating system. The total nucleotide (bound and free) remained 86% ATP, but isolated GA* contained only 37% ATP and the rest of the nucleotide as ADP. On the assumption that no nucleotide exchange occurred, the ATP in the complex had a half life of about 20 h (about 40 h in a second experiment).

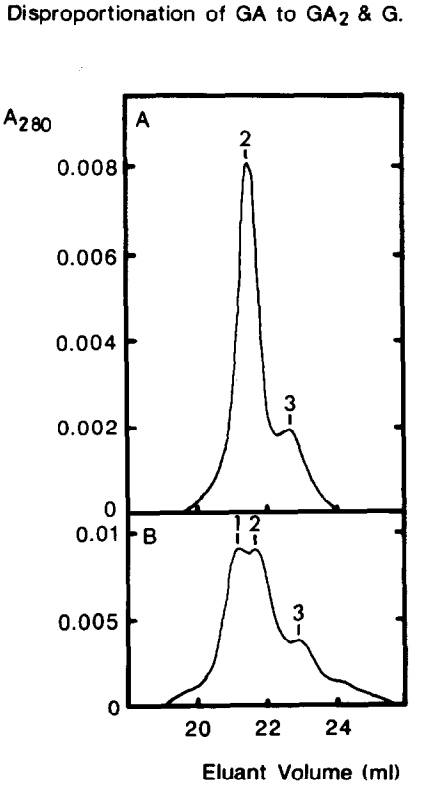

Fig.1. Disproportionation of GA* to GA_2 and gelsolin in excess Ca^{2+}. (A) Mixture of 3.3 μM gelsolin with about 3 μM actin to give GA* (with a trace of free gelsolin); 5 mM imidazole chloride, pH 7.0, 2 mM $MgCl_2$, 150 mM NaCl, 0.1 mM EGTA, 1 mM sodium azide. (B) Chromatographically purified GA* incubated 72 h in 1 mM $CaCl_2$, and chromatographed in 0.1 mM $CaCl_2$. Peaks: 1, GA_2; 2, GA*; 3, gelsolin.

Lack of nucleotide exchange from GA* was supported by overnight incubation of GA* containing [^{3}H]ATP in 1 mM unlabelled ATP; only 12.6% of the bound nucleotide was displaced (table 1). Lower protein concentrations gave greater nucleotide displacement, indicative of GA* dissociation. The proportions of displaced (free) and protein-bound nucleotide were determined following HPLC analysis, for a range of GA* dilutions (table 1). The same GA preparation,

chromatographed at 5.5 μM with no added ATP, lost only 6.9% of its nucleotide. The proportion of nucleotide displaced decreased with increasing protein concentration.

GA* prepared from Mg-G-actin-ADP contained 1.02 ± 0.11 (n = 3) mol ADP per mol GA* prepared at 7 μM. When this GA* containing [^{3}H]ADP was diluted and incubated in the presence of unlabelled ATP (0.5 μM GA* in 2.5 mM ATP), it lost 45% of the bound [^{3}H]ADP in 24 h and 70% in 72 h; the HPLC elution profile of the same samples showed partial dissociation to gelsolin and actin. Thus GA* prepared with either ATP or ADP appears to dissociate at high dilution.

3.3. *Disproportionation of GA* at high Ca^{2+}*

GA* was incubated in 1 mM Ca^{2+} (10-fold excess over EGTA). Analysis on HPLC (fig.1B) showed maxima for both GA* and GA_2, together with a small peak of free gelsolin. GA_2 is formed from the reaction: 2GA = GA_2 + G.

3.4. *Actin-bound nucleotide in GA_2 formed from Mg^{2+}-actin*

GA_2 was prepared under the same conditions as GA*, except that two actins were added per gelsolin and the buffer contained 2 mM $MgCl_2$ and 20 μM free Ca^{2+} to give Ca^{2+}-gelsolin and Mg^{2+}-actin. ATP- and ADP-actins gave complexes containing 1.7 and 1.8 mol nucleotide per mol of complex, respectively; about half of this nucleotide was displaced by unlabelled ATP (0.5 mM for 16 h) from both ATP (56.0%) and ADP (55.7%) containing complexes. GA_2 forms very rapidly, but within 10 min of mixing stoichiometric gelsolin and actin, no significant ATP cleavage had occurred. Thus hydrolysis is not coupled to complex formation. However, up to half the nucleotide was hydrolysed during isolation of the complex (about 3 h). In two experiments, 54 ± 4% of the ATP in the complex was hydrolysed to ADP by the time of isolation, but no further hydrolysis occurred on prolonged incubation (49 ± 1% cleaved after 21 h, 0°C).

4. DISCUSSION

The binary complex here described forms from actin-ATP or actin-ADP. It thus resembles GA_2 [28]. The formation requires physiological ionic strength and therefore takes place only under actin polymerizing conditions. However, it was shown with actin-ADP that GA* forms below the critical concentration, and direct interaction of gelsolin with actin monomers is therefore likely.

GA* has been prepared from pig plasma gelsolin ([5] and this paper) but did not form from bovine plasma [4,6] or cytoplasmic (platelet) [7] gelsolins. Binary complex formation may be a specific property of pig gelsolin. Alternatively, it may be critically dependent on ionic conditions, since gelsolins from other sources were tested at low ionic strength [4,7] or in the absence of divalent cations [6].

Gelsolin binding to Mg^{2+}-G-actin-ATP inhibited both nucleotide hydrolysis (about 5–10-fold relative to free monomer [29]) and nucleotide exchange. Exchange was also inhibited in GA′ containing trapped calcium ions [30]. The limited data on GA_2 prepared from Mg^{2+}-actin are consistent with a model with one enzymatically active actin exhibiting nucleotide exchange and one inert site. The latter may be equivalent to the site in GA*. The model for GA_2 is supported by [30], in which

Table 1

Displacement of bound ATP from GA*: dependence on protein concentration

	GA* concentration (nM)	% nucleotide displaced	Recovery (cpm per μl sample)
Expt 1	360	22.0	–
	900	12.6	–
Expt 2	82.5	57.2	907.9
	192.5	42.4	742.5
	275	36.0	745.8
	550	26.6	724.2
	1100	25.5	664.8
	2750	20.1	756.5
	5500[a]	6.9[a]	–

[a] No unlabelled ATP present

GA* was prepared containing ^{3}H-ATP, and subsequently incubated at various dilutions for 24 h, 0°C, in the presence of 1 mM unlabelled ATP. Protein-bound and displaced (free) radiolabelled nucleotide were separated by HPLC and quantitated. Total recovery of radioactive material was 757 ± 74 (n = 6) cpm per μl. Proportionately lower recoveries of protein-bound counts at low protein concentrations were therefore not an artifact of preferential loss of protein by adsorption and can be explained only by GA* dissociation

it was shown that the ternary complex hydrolyses ATP, possibly at a single actin site, and that one site in GA_2 did not exchange nucleotide. (There is a discrepancy with Coue and Korn [31], who did not observe nucleotide exchange from GA_2.)

Actin binding to gelsolin in GA* is weaker than that of actins in GA_2. The dependence of nucleotide displacement on GA* concentration, shown in table 1, implies dissociation of the binary complex. In addition, evidence for dissociation could be seen in HPLC column profiles. By contrast, displacement of nucleotide from GA_2 was about 50%, independently of ATP concentration, protein concentration (above 100 nM) or incubation time, implying lack of any exchange at the second ATP site [21], and therefore lack of dissociation of the proteins. The dissociation of GA* is consistent with the K_d in the range of 30–60 nM obtained by forward titration of fluorescent actin with gelsolin [5]. Some nucleotide may also be displaced from undissociated GA*. This possibility is suggested by the loss of 20% of the nucleotide at a GA* concentration of 50–100-fold above K_d (table 1). Further data on the extent to which nucleotide loss is coupled to GA* dissociation are needed to confirm this suggestion.

The relatively weak binding of gelsolin to actin in GA*, and the partial disproportionation of GA* into GA_2 and free gelsolin (fig.1), are consistent with cooperative formation of GA_2 [8].

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